ESTABLISHMENT AND CLONAL PROPAGATION OF *IN VITRO* PLANTLETS OF *LEPTOSPERMUM SCOPARIUM*

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ABSTRACT

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Nodal segments of manuka (*Leptospernum scoparium*, J.R. *et* G. Forst.) were surface sterilised and initiated into aseptic culture. Optimal conditions for culture establishment were investigated. Explants grew well on low salt media without the inclusion of growth factors or carbon source and rooting occurred readily in such conditions. The presence of auxins had no significant enhancing effect on root formation or elongation compared to control. Cytokinins promoted bud formation but inhibited their elongation. *In vitro* plantlets could be readily multiplied. Roots formed were shown by sectioning to be of pericycilc origin.

KEYWORDS: Manuka - Tissue culture - micropropagation - *Leptospermum scoparium* - nodal explant - New Zealand - native plant.

Abbreviations : NAA, Napthalene acetic acid; IBA, Indole 3-butyric acid; NOA, B-Napthoxy acetic acid; 2,4-D, 2,4-Dichlorophenoxy acetic acid; BA, Benzyl adenine; GA, Gibberellic acid.

INTRODUCTION

Leptospermum scoparium (J.R. *et* G. Forst.), commonly known as manuka, is a widely distributed New Zealand shrub of the family Myrtaceae. It is an important successional plant on open lands and it has the capacity to stabilise eroded hill country slopes. Traditionally, it was known for various medicinal uses as outlined by Brooker *et al.* (1987). The essential oil of manuka contains the ketonic compound leptospermone which has some antibacterial activity (Atkinson & Brice 1955) and other properties of commercial potential (Brooker *et al.* 1988). The species is horticulturally important as many cultivars have been produced (Metcalf 1987) and overseas exports of some of these as "mini potplants" have been investigated by the Ministry of Agriculture and Fisheries (1983/1984). Three Australian species of *Leptospermum* and the Australian bottlebrush *Callistemon viminalis*, all from the subfamily Leptospermidae, are related species that have successfully been micropropagated (Shipton 1982, Shipton & Jakes 1986). Propagation of manuka may be achieved by rather bulky semi-hardwood cuttings or seeds (Metcalf 1987). However micropropagation has the potential for rapid cloning of a desired cultivar or a clone with high essential oil content. Therefore, the requirements for *in vitro* culture of manuka were investigated.

MATERIALS AND METHODS

PLANT MATERIAL

Young plants, approximately two years old were grown from seed collected from Otaio, South Canterbury by the Department of Conservation nursery at Motukarara. Nodal sections, and later apical tips from portions of new growth were used as explants.

TISSUE CULTURE

Cuttings two to four centimeters long were soaked in a 1% solution of sodium hypochlorite and then washed three times in sterile distilled

water before being trimmed to a suitable length in a laminar flow cabinet. The basal medium consisted of 1/10 strength of inorganic salts (Murashige & Skoog 1962), without addition of vitimins, growth factors or carbon source as recommended by Burritt and Leung (1991) for *in vitro* propagation of *Hebe speciosa*. The pH of all media was adjusted to 5.6-5.8 and then 8g/l agar (Davis) was added before sterilisation in an autoclave for 14 mins at 137 KPa pressure. When required, the appropriate auxins and cytokinins were incorporated into the media before sterilisation. GA_3 was filter sterilised using 0.2 µm Millipore filters and added to the media following sterilisation. Explants were placed in 50 ml clear plastic tissue culture vessels of height 70 mm and diameter 50 mm, each containing 20 ml of the appropriate media. All cultures were incubated in a culture room with constant temperature of about 23°C with 24 h lighting at 125 $\mu Em^{-2}s^{-1}$. Root and stem junctions were embedded in wax and sectioned before staining with fast green and safranin.

RESULTS AND DISCUSSION

As a preliminary trial, success of initiation into media containing various plant growth regulators was assesed (Table 1). These initial trials showed moderate concentrations of NAA or hormone free media produced the most consistent results, promoting rooting, branching and shoot elongation (Fig. 1a). Explants on BA media were stunted and rootless, although a few produced small roots when transferred to media containing 0.1 mg/l NAA (Fig. 1b). The browning and swelling of such explants could explain the low percentage of rooting upon removal from BA. Explants on GA_3 containing media were spindly and suffered from chlorosis.

A differing response depending on the explant type was observed. Single node explants were often damaged by excision from the stem and became dormant. If placed on NAA or hormone free media they were revived after 3-4 months and grew as vigorously as other types of explant. On BA/NAA combination media single node explants typically died. The order of responsiveness of explants was as follows:

single node < multiple node < multiple node and apical (semihardened) < multiple node and apical (unhardened).

Therefore newly grown shoot tips were used preferentially in subsequent experiments. Semihardened shoots gave similar end results over

Table 1. Relative growth response of nodal explants of greenhouse grown manuka to plant growth regulators after 12 weeks in culture[a].

Treatment (mg/l)		Formation of roots	Formation of shoot buds	Elongation of shoots
control		+++	++	++
NAA	0.1	+++	++	++
	0.2	+++	++	++
BA	1.0	-	+++	-
	2.0	-	+++	-
	5.0	-	+[b]	-
GA_3	1.0	++	+	+++[c]
	5.0	++	+	+++
	10.0	+	+	+
BA&NAA combinations[d]		-	-	-

[a] +++ = good formation, ++ = adequate formation, + = slight formation, - = no formation.
[b] This concentration promoted explant death.
[c] Shoots bolted and could not support themselves.
[d] From a factorial trial of 1.0, 2.0 or 5.0 mg/ml BA combined with 0.1, 0.2, 0.5, 1.0 or 2.0 mg/ml NAA.

Figure 1. The effect of auxins and cytokinins on explant growth. a) shows the amount of growth achieved after three months on media containing 0.1mg/l NAA. b) shows a similar explant after two months on 2mg/l BA followed by one month on 0.1 mg/l NAA. Approximately full size.

a longer time period. Trimming of parental plants was useful in stimulating new growth as observed by other workers (Das & Mitra 1990).

To establish which auxin if any had the greatest stimulatory effect on growth of the explants, different auxins and some varying concentrations of NAA were tested. Table 2 shows the growth response of apical explants excised from greenhouse grown plants which included 2 to 3 nodes, and apical shoots from *in vitro* plantlets. From Table 2 it can be seen that explant growth was not adversely effected by being kept free from any of the growth regulators tested, i.e. no individual auxin had a pronounced stimulatory effect. Explants that had been conditioned to culture conditions showed enhanced growth over explants from unacclimitised sources.

Results of the histology analysis shows the adventitious roots of *in vitro* manuka plantlets were originating endogenously, in a manner similar to lateral roots, indicating functional roots are being formed (Fig. 2).

The methods presented here show a simple and reliable way of multiplying manuka *in vitro*, enabling the availability of a year round supply of tissue for propagation. The ability of manuka to grow on media free of hormones, growth factors and a carbon source, as well as eliminating the need for independent rooting and initiation steps, greatly simplifies the process. Planting out experiments are currently underway although problems are being experienced with microbial contamination.

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Table 2. Relative effects of various auxins and auxin concentrations on the growth of apical explants of greenhouse grown manuka and on apical explants of *in vitro* grown manuka after four weeks in culture.[a]

Treatment (mg/l)		Formation of roots	Formation of shoots	Elongation of shoots
Greenhouse grown explants				
control		+ +	+ +	+ +
NOA	0.1	+ +	+ +	+ +
IBA	0.1	+ +	+ +	+ +
2 4-D	0.1	+ +	+ +	+ +
NAA	0.1	+ + +	+ +	+ +
	0.2	+ +	+ +	+ +
	0.5	+ + +	+	+
In vitro grown explants				
control		+ +	+ + +	+ + +
NAA	0.1	+ +	+ + +	+ + +

[a] + + + = maximum growth observed, + + = good growth, + = moderate growth.

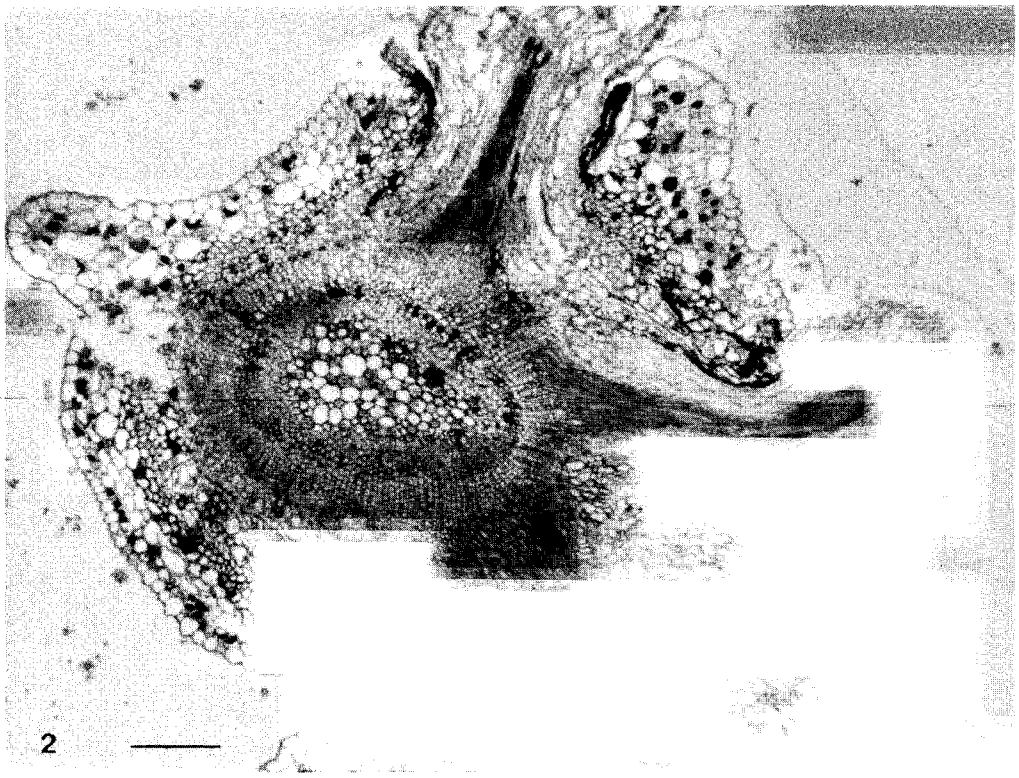

Figure 2. Cross section of a manuka plantlet where new roots have arisen from the base of the explant. Bar = 0.2 mm.

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